PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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2340050 - 26/01/2005
JL

PCT

WRITTEN OPINION
(PCT Rule 66)

(day/month/year) 24.01.2005

Applicant's or agent's file reference	REPLY DUE	
PCT-154		within 3 month(s) from the above date of mailing

International application No.	International filing date (day/month/year)	Priority date (day/month/year)
PCT/ES2004/070001	21.01.2004	28.01.2003

International Patent Classification (IPC) or both national classification and IPC
C12N15/09

Applicant
EFARMES S.A. et al.

1. This written opinion is the **first** drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☒	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☐	Certain observations on the international application

3. The applicant is hereby **invited to reply** to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also: For an additional opportunity to submit amendments, see Rule 66.4. For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 bis. For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 28.05.2005

Name and mailing address of the international preliminary examining authority:

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I. Basis of the opinion

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed")*:

Description, Pages

1-61 as originally filed

Sequence listings part of the description, Pages

1-91 as originally filed

Claims, Numbers

1-16 as originally filed

Drawings, Sheets

1/3-3/3 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: English , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   ☐ the language of publication of the international application (under Rule 48.3(b)).
   ☒ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.
   ☐ filed together with the international application in computer readable form.
   ☐ furnished subsequently to this Authority in written form.
   ☒ furnished subsequently to this Authority in computer readable form.
   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:
   ☐ the claims, Nos.:
   ☐ the drawings, sheets:

5. ☐ This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

6. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation (Form PCT/IPEA/405) to restrict or pay additional fees, the applicant has:

   ☐ restricted the claims.

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied with for the following reasons and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees:

   see separate sheet

3. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this opinion:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	
Inventive step (IS)	Claims	1-16
Industrial applicability (IA)	Claims	

2. Citations and explanations

   see separate sheet

Present application relates to *in vitro* methods for detecting individuals who are predisposed to the disease named Familial Hypercholesterolemia (FH), and more particularly to a method for detecting the presence or absence of several mutations associated with FH. 54 gene mutations and polymorphisms that are all produced in the gene sequence of the low density lipoprotein receptor gene (LDL-r) as set forth in SEQ ID NO:1 are disclosed. Sequences characterised by said mutations, use of said sequences as well as oligonucleotides hybridising with any of the mutations comprised in said sequences and the use of said oligonucleotides are claimed.

Re Item IV

Lack of unity of invention

The present application appears to lacks unity within the meaning of Rule 13.1 PCT. The following separate inventions can be considered:

a) Invention 1 (Claims 1 - 10 and 12 - 16; all partially):

Claims 1 - 10 and 12 - 16 relate to a gene sequence corresponding to SEQ ID NO:1 comprising the mutation (-23)A> C and the subject matter relating to said gene sequence.

b) Inventions 2 - 54 (Claims 1 - 10 and 12 - 16; all partially):

As for Invention 1, but respectively relating to the mutations 1054del11, 108delC,.....[1587-5del15;1587del31] (i.e. Invention 2 corresponding to the mutation 1054del11; Invention 3 corresponding to the mutation 108delC.....; Invention 54 corresponding to the mutation [1587-5del15;1587del31]) and the subject matter relating to said gene sequences.

c) Invention 55 (Claim 11):

Claim 11 relates to the use of 33 oligonucleotides in an extracorporeal method of in vitro detection of LDL-r gene mutations for the diagnosis of FH.

The 55 inventions are not so linked as to form a general inventive concept for the following reasons:

The problem to be solved by the present application can be seen as provision of gene sequences comprising mutations in the LDL-r gene and the use of said sequences in *in vitro* methods for the detection of FH as well as the use of the oligonucleotides set forth in claim 11 for the *in vitro* detection of FH. The solution to this problem is provided with the gene sequences comprising any of the mutations as set forth in claim 1, respectively the oligonucleotide sequences as set forth in claim 11.

However, this general concept is "*a posteriori*" not novel, since a number of LDL-r gene mutations in FH had been publicly known before the priority date of the present application (see ISR: e.g. D1: page 3, line 21 - page 5, line 13; D2: Table 1). Also, it had been publicly known before the priority date of the present application to detect lipid metabolic errors such as FH by noting these mutations.
Therefore and since no other technical feature can be distinguished which might link the subject matter of said claims, each of the above mentioned group of claims represents an independent invention.
Hence, the present application does not meet the requirements of unity of invention as defined in Rules 13.1 and 13.2 PCT.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

V.1 The following documents were taken into account:

D1: WO 02 06467 A1, respectively EP1304374

D2: FOUCHIER S. ET AL: 'The molecular basis of familial hypercholesterolemia in The Netherlands.' HUMAN GENETICS vol. 109, no. 6, December 2001, pages 602 - 615, XP002980736

The European Patent Application EP1304374 which has been published in accordance with Article 158(3) EPC corresponds to the PCT application WO0206467 published on the 24.01.2002. Hence, EP1304374 is validly considered as English translation of WO0206467.

V.2 Inventive Step (Article 33(1) and (3) PCT)

V2.1 The technical contribution of the present application can be summed up as the provision gene sequences comprising mutations in the LDL-r gene as set forth in claim 1 and the use of said sequences in *in vitro* methods for the detection of FH. With regard to prior art (D1 and D2 already disclose mutations in the LDL-r gene and methods for the detection of the same; D1: page 3, line 21 - page 5, line 13; D2: Table 1), the technical problem solved by the present application is the provision of alternative mutations of the LDL-r gene.
The 54 mutations as referred to in claim 1 are found out merely by comparing the base sequence of the normal LDL-r gene with the base sequences of LDL-r genes of patients clinically diagnosed as suffering form FH. Hence, taking into consideration the prior art in combination with general knowledge, the provision of further gene sequences comprising mutations in the LDL-r gene would be obvious

and straight forward for the person skilled in the art. The IPEA raises the Applicant's attention to the fact that even some of the claimed mutations are already suggested by the prior art, for example: D1 refers to a mutation of the LDL-r gene occurring at a site coding for the amino acid residue 74 (page 43, lines 7 and 8).
In addition, in order for inventive step to be recognised for alternatives to known products and methods, it is necessary that the claimed alternatives provide a technical effect different from the prior art. The technical effect must also be described in precise terms. Therefore, to establish an inventive activity, the provision of further mutations must be justified by the technical purpose, i.e. by a hitherto unknown or unexpected effect, caused by those technical features which distinguish the claimed molecule form numerous other ones. Thus, it is not possible to acknowledge inventive step for the application, as no technical effect of the claimed polynucleotides over prior art is revealed. Hence, claims 1- 10 and 12 - 16 are not considered to be inventive under Article 33(3) PCT.

2.2 Claim 11 refers to the use of oligonucleotides in an *in vitro* method for the detection of FH, and more precisely claim 11 discloses amplification primers that are used for the amplification of the LDL-r gene. Since the LDL-r gene as well as methods for the detection of mutations in said gene are well described in the prior art, the provision of amplification primers that are directed to the LDL-r gene lies within customary practice of the person skilled in the art and does not involve an inventive step. Hence, the subject-matter referred to in claim 11 does not appear to fulfill the requirements of Article 33(3) PCT.

Certain Observations on the International Application

*The following remarks on **Clarity and Sufficiency of Disclosure** (Article 6 and 5 PCT) are made:*
*1) Claims 4 and 5 do not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined (**...capable of hybridising....**). The claims attempt to define the subject-matter in terms of the result to be achieved, which merely amounts to a statement of the underlying problem, without providing the technical features necessary for achieving this result. Moreover, the term "hybridising", without the indication of the hybridisation conditions, is vague and unclear and leaves the reader in doubt as to the meaning of the technical feature to which it refers, thereby rendering the definition of the subject-matter of said claims unclear, Article 6 PCT.*

2) With regard to the description, page 57, lines 12 and lines 14, 22 and 27 it is unclear whether the mutation 2389+3C>A or 2389+3C>T is correct. Hence, the subject-matter referred to in claim 1 does not fulfill the requirements of Articles 5 and 6 PCT.